The Antibiotic Resistance of Bacterial Pathogens Isolated From Poultry Manure Used For Fish Ponds

Veterinary Medicine

Keywords: Pathogenic bacteria, antibiotic resistance, sensitivity, poultry manure.

Dritan Laçi	**Agricultural University of Tirana, Faculty of Veterinary Medicine, Department of Veterinary Public Health**
Bejo Bizhga	**Agricultural University of Tirana, Faculty of Veterinary Medicine, Department of Veterinary Public Health**

Abstract

This study was carried out to isolate and identify antibiotic resistant bacteria from poultry manure usually used for pond fertilization. We collected poultry manure which used in Experimental Center for Fisheries Research, Tapize (ECFR), Agricultural University of Tirana in 2012. During the study we have isolated five bacterial pathogens; Salmonella typhi, Escherichia coli, Shigella dysenteriae, Staphylococcus aureus and Aeromonas hydrophila. Antibiotic susceptibility testing carried out using the disk diffusion technique. Antibiotics used were: enrofloxacin, amoxicillin, tetracycline, ampicillin, erythromycin and gentamicin. All the isolated organisms were 100% sensitive to enrofloxacin. The multiple resistance pattern revealed that 100% were resistant to tetracycline, 84% resistant to ampicillin, 81% resistant to amoxicillin, 66% resistant to gentamicin and 23% resistant to erythromycin. The risk posed by untreated poultry manure used in fish pond fertilization and the public health implications of these results were discussed.

1. Introduction

Animal manure is used traditionally used to fertilize fish ponds. The pond water becomes fertile upon the application of manure, resulting in more food organisms, thus a high fish production, (Din, Jie-yi and Guo, Xianzhen, 1988). The use of untreated livestock manure releases high concentrations of pathogenic microorganisms into the ponds constituting a high risk to fish and fish farmers, (Prithwiraj Jha, *et al* 2008), reported that average counts of heterotrophic bacteria were significantly higher in the pond water on the application of livestock manure. The development of *Pseudomonas* and *Aeromonas* were also significantly higher ($P<0.05$). This has grave consequences as some of these bacteria have been reported to be resistant to conventional antibiotics, (Olaitan JO, *et al*., 2011). Antibiotics and other antibacterial drugs are the major weapons against disease-causing bacteria. They act in a number of ways to kill bacteria or suppress their activity. Over time, however, bacteria can become resistant to antibiotics. Extensive use and misuse of antibiotics in medication, veterinary, agriculture and aquaculture have increased the occurrence of antibiotic resistance bacteria, (Kummerer K, 2004), in the natural environment. Resistance genetic material transfer from environmental bacteria to commensal microflora may also cause bacterial pathogens to carry antibiotic resistance, complicating disease prevention and treatment (Kummerer K, 2004; Levy SB and Marshall. B, 2004). In countries which are in development processes, antibiotics are sold over the counter without a prescription which compounds the problem. In human medicine, the major problem of the emergence of resistant bacteria is due to misuse and overuse of antibiotics by doctors as well as patients (Witte. W, 1998). Other practices contributing towards resistance include the addition of antibiotics to feeds of livestock (Matthew AG, *et al*., 2007). Antibiotic-resistant organisms in domestic animals such as poultry, beef and swine are well documented (Prithwiraj Jha, *et al*. 2008) and have been implicated as reservoirs for multidrug-resistant food borne pathogens. Also unsound practices in the pharmaceutical manufacturing industry such as production of counterfeit drugs can contribute towards the likelihood of creating antibiotic resistant strains (Larson DG and Fick J., 2009). Emergence of bacteria resistant to antibiotics is common in areas where antibiotics are used, but occurrence of antibiotic resistance bacteria is also increasing in freshwater basins (Sarten. S, *et al*., 2007). However, relatively very few studies on antibiotic resistant bacteria have been carried out on dumpsite soils, water sources, and duck droppings in the environment and hospital environment (Ikpeme. E, *et al*., 2011; Olaitan. J. O, *et al*., 2011; Omololu-Aso J, *et al*., 2011) and even fewer studies have been undertaken in dynamic integrated aquaculture environment where manures are usually used to fertilize fish ponds.Therefore, the present study was carried out to isolate and identify antibiotic resistant bacteria from poultry manure used to fertilize fish ponds in fisheries environment and also to determine their antibiotic susceptibility patterns.

2. Material and Methods

2.1 Sample collection: Faecal droppings of chicken were collected randomly from us in ECFR, Tapize, Agricultural University of Tirana. A total of ten samples were collected from each 130 chickens, from the farm that helps the pond fertilization. The faecal droppings were collected with sterile spatula into sterile peptone water. All samples were analyzed within 1 hour of collection.

2.2 Isolation and identification: Isolation and identification of bacteria were investigated according to the manual of (Holt. GH, *et al*., 1997).

2.3 Antibiotic Sensitivity Testing: Antibiotic resistance of bacteria was determined by the single disc diffusion method with the use of Mueller-Hinton agar, according to the Bauer-Kirby method, (Bauer. A. W, *et al*., 1966). The following eight clinical antibiotics, with their concentrations given in parentheses were used in the antibiograms as recommended by the Performance Standards for Antimicrobial Susceptibility Testing (2007): tetracycline (30μg), enrofloxacin (30μg), gentamicin (20μg), erythromycin (10μg), ampicillin (10μg) and amoxicillin (30μg). After incubation, a clear circular zone of no growth in the immediate vicinity of a disk indicates susceptibility to that antimicrobial. Using reference tables the size of zones was related to the Minimum Inhibitory Concentration (MIC) and results recorded as whether the organism is susceptible (S) or resistant (R) to that antibiotic. Data were statistically analyzed using SPSS Version 12.

3. Results and Discussion

The occurrence of the 144 isolates from Poultry manure (Fig.1) revealed that 62 (43 %) were *Escherichia coli*, 24 (17%) were *Aeromonas hydrophila*, 22 (15%) were *Salmonella typhi*, 19 (13%) were *Shigella dysenteriae* and 17 or 12% were *Staphylococcus aureus*. The isolates were mostly enteric organisms.

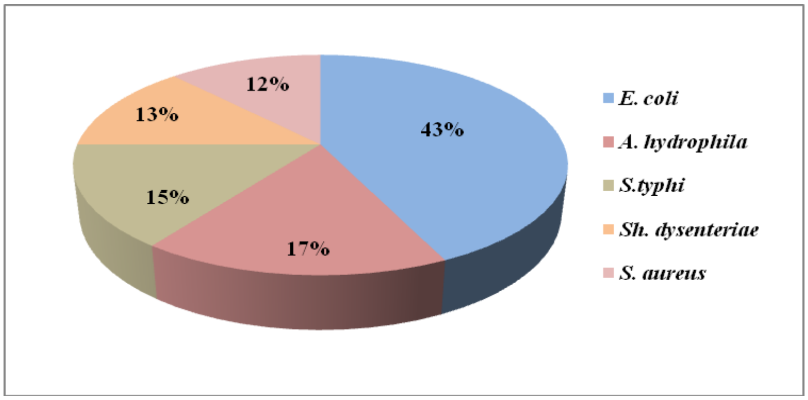

Figure 1. Frequency of occurrence of isolated organisms in poultry manure to fertilize fish ponds.

Salmonella typhi had 100% susceptibility to enrofloxacin and 75% to erythromycin. However the same isolates had 100% resistance to tetracycline and 75% to resistance to ampicillin, amoxicillin and gentamicin (Fig. 2).

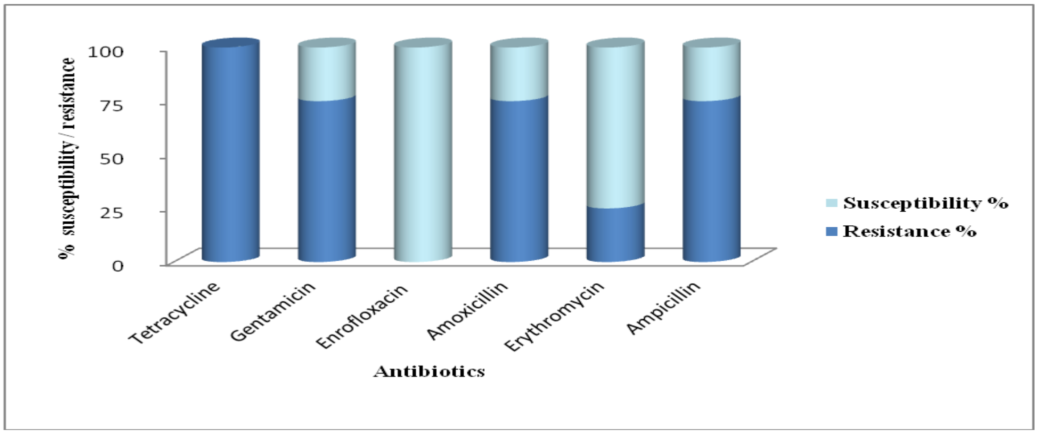

Figure 2. Antibiogram of *Salmonella typhi* isolated from poultry manure used to fertilize fish ponds.

Escherichia coli recorded 100% susceptibility to enrofloxacin and 80% susceptibility to erythromycin. However, it had 100% resistance to amoxicillin and tetracycline; 80% resistance to gentamicin and ampicillin respectively (Fig. 3).

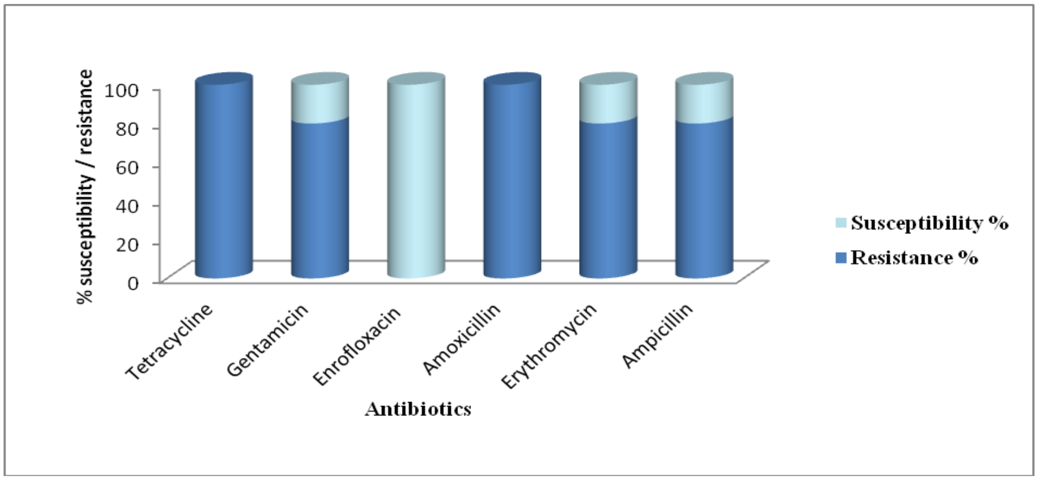

Figure 3. Antibiogram of *Escherichia coli* isolated from poultry manure used to fertilize fish ponds.

Fig. 4 shows *Shigella dysenteriae* with 100% susceptibility to enrofloxacin and erythromycin; with 100% resistance to tetracycline and ampicillin respectively. It also had 65% resistance to amoxicillin and gentamicin.

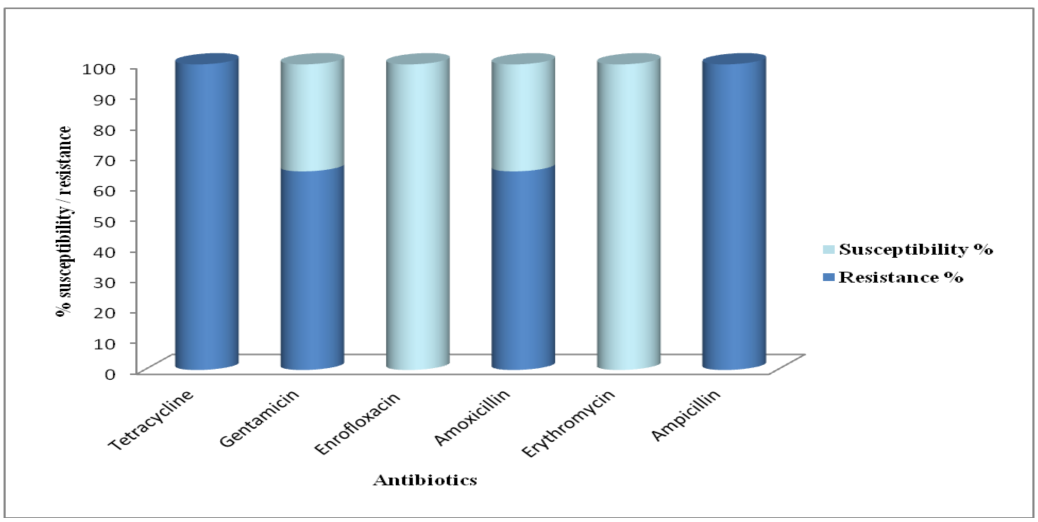

Figure 4. Antibiogram of *Shigella dysenteriae* isolated from poultry manure used to fertilize fish ponds.

Aeromonas hydrophila was 100% susceptible to enrofloxacin; with 100% resistance to tetracycline, amoxicillin and ampicillin respectively. In following was 90% resistance to erythromycin and had 75% resistance to gentamicin, (Fig.5).

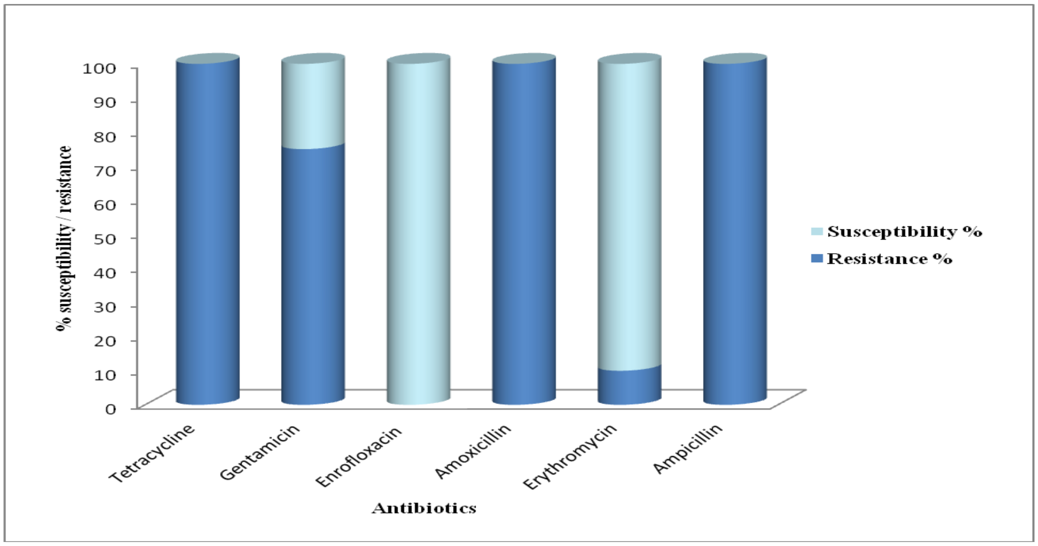

Figure 5. Antibiogram of *Aeromonas hydrophila* isolated from poultry manure used to fertilize fish ponds.

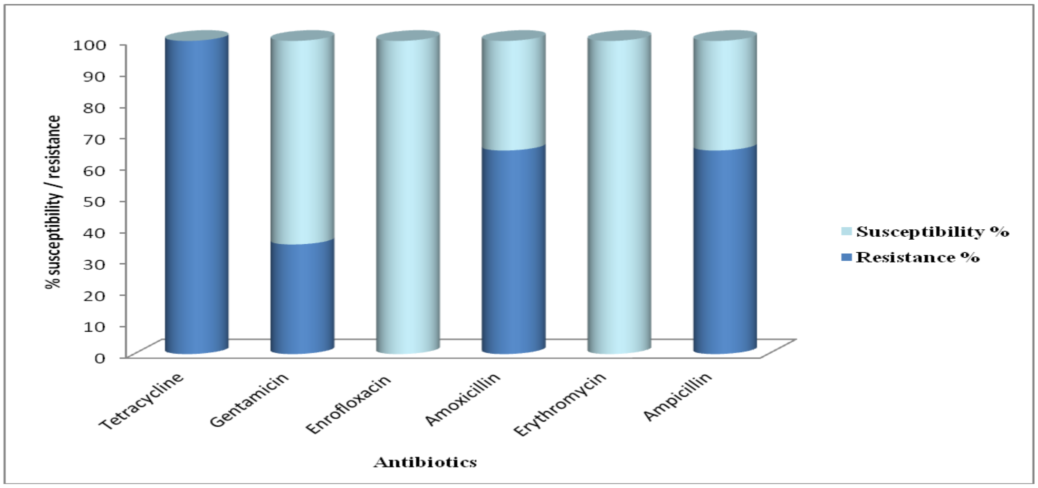

Figure 6. Antibiogram of *Staphyloccocus aureus* isolated from poultry manure used to fertilize fish ponds.

Staphylococcus aureus recorded 100% susceptibility to erythromycin and enrofloxacin respectively and gentamicin 65%. However, it had 100% resistance to tetracycline, 65% to amoxicillin and ampicillin respectively.

Overall, there was a 100% resistance to tetracycline by all the isolates, 84% resistant to ampicillin, 81% to amoxicillin, 66% to gentamicin, 23% to erythromycin. There was 0% resistance to enrofloxacin by all the isolates (Fig. 7).

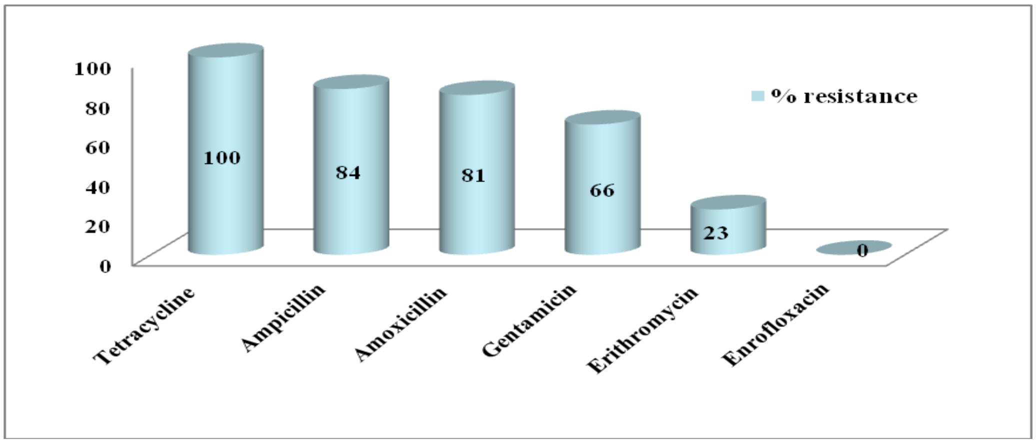

Figure 7. Antibiotic multiple resistance pattern of bacteria isolated from poultry manure for fertilizing fish ponds.

The results of this study show that the antibiotics tetracycline recorded 100% resistance from all the bacterial isolates, >75% resistance for ampicillin, >70% resistance amoxicillin, >55% resistance gentamicin and <30% resistance for erythromycin. All the bacteria isolated had 0% resistance to enrofloxacin. This trend is quite worrisome as these antibiotics represent first line antibiotics in the treatment of many bacterial infections (Omololu-Aso. J, *et al*., 2011). Resistance of a single bacterial isolate to more than one antimicrobial drug is commonly reported. Multiple antimicrobial drug resistance profiles have been reported (Ikpeme. E, *et al.*, 2011). This type of testing is simple, cost-effective, and suitable for surveillance and it has been used for enteric bacteria from human and animal sources (Troy. MS, *et al*., 2002).

Results from this study show *Escherichia coli* had a 100% resistance to tetracycline and amoxicillin; 80% resistance to ampicillin, gentamicin and erythromycin respectively. For the tetracycline and amoxicillin this is similar to the report (Aibinu. I, *et al*., 2004), who reported 100% resistance of their *E. coli* isolates. This study also observed 100% susceptibility of *Escherichia coli* to enrofloxacin. This is also similar to the report of (Olaitan. JO, *et al*., 2011), who recorded 100% susceptibility to enrofloxacin. The high level of resistance of *Shigella dysenteriae* to tetracycline and ampicillin reported in this study was similar to the report of (Ikpeme. E, *et al*., 2011) who reported resistance to tetracycline and ampicillin. The susceptibility recorded by *S. aureus* in this study for enrofloxacin and erythromycin were 100% respectively and is however slightly higher than the susceptibility reported by (PSAST 2007) for erythromycin which were 73.98%. However the susceptibility to gentamicin reported was 65% which was slightly higher than those reported in this study. The patterns of resistance to the antimicrobial agents may be due to indiscriminate, widespread and lengthy use of tetracycline and gentamicin in poultry. Tetracycline is a commonly used first-line antibiotic in the poultry and is often used before the antimicrobial agent resistance of a pathogen has been determined (Giguere. S, *et al*., 2013). The highest levels of susceptibility to all bacterial isolates found in this study were to enrofloxacin and erythromycin (Fig. 6), is in line with the findings of (Engberg. J, *et al*., 2001; Tricia D. Miles**,** *et al.,* 2006).

4. Conclusions

The results of this study indicate that poultry manure is a potential carrier of pathogenic bacteria which is capable of transmitting zoonotic diseases to humans due to contact with the manure, pond water and the fish from the pond. These antibiotic resistant organisms may be further transferred to consumers in improperly processed fish. The antibiotic sensitivity testing show that all the organisms were 100% sensitive to enrofloxacin whereas the isolates were most resistant to antibiotics like, tetracycline, ampicillin, amoxicillin and gentamicin which are the common antibiotics in treating bacterial infections in both man and animals. This study further revealed that fish, fertilized fish ponds and the environment are easily contaminated by poultry manure intended for fish production; therefore it is important to use untreated animal manure with caution. The implication of this on the choice of antibiotics in relation to zoonotic infections in our environment should be noted and effort should be made to stop indiscriminate use of untreated animal manure for fish pond fertilization.

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